



Improvement in growth parameters and essential oil content and characteristics of populations derived from intraspecific crosses of *Mentha spicata* and *M. longifolia*

Hamid Yaghini · Mohammad R. Sabzalian · Mehdi Rahimmalek · Negar Ziaei

Received: 19 May 2020 / Accepted: 13 February 2021 / Published online: 8 March 2021
© The Author(s), under exclusive licence to Springer Nature B.V. part of Springer Nature 2021

Abstract Populations derived from intraspecific crosses within two mint species of *Mentha spicata* and *M. longifolia*, were studied for genetic and phenotypic coefficients of variation, heritabilities, and genetic advances in various traits including plant height, fresh and dry weights, number of branches per plant, plant canopy diameter, leaf area, and essential oil content and yield. The majority of agro-economic characters showed high heritability (h^2) and genetic advances in the F_2 generation. The maximum genetic advance and genotypic coefficient of variation were recorded for fresh weight, leaf area, and essential oil content and yield. Based on these traits, total flavonoid (TFC) and phenolic (TPC) contents of the best genotypes from each species of *M. longifolia* and *M. spicata* were estimated. The results showed that some of the F_2 plants had a higher amount of TFC and TPC

compared with the parents. The analysis of essential oil components also revealed that the amount of piperitenone and menthone in *M. longifolia* and carvone and limonene in *M. spicata* significantly increased in the selected genotypes derived from intraspecific crosses. It is hypothesized that the improvements in the offspring derived from intraspecific crosses of mint are attributed to transgressive segregation and heterosis. Therefore, crossing within mint species can be effectively utilized for recombination breeding to obtain genotypes with a novel genetic structure potentially usable in breeding programs for pharmaceutical purposes.

Keywords Intraspecific cross · Segregating generation · Heterosis · Essential oil · Mint

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10681-021-02787-y>.

H. Yaghini · M. R. Sabzalian (✉) ·
M. Rahimmalek · N. Ziaei
Department of Agronomy and Plant Breeding, College of
Agriculture, Isfahan University of Technology,
Isfahan 84156-83111, Iran
e-mail: sabzalian@iut.ac.ir

M. Rahimmalek
Department of Horticulture, College of Agriculture,
Isfahan University of Technology, Isfahan 84156-83111,
Iran

Introduction

Mint (*Mentha* spp.) is one of the main taxa belonging to the Lamiaceae family (Benabdallah et al. 2016), which is planted in warm, Mediterranean, and subtropical regions. This genus is classified into five sections, including various hybrids and approximately 30 species. Mints are generally used as a vegetable and for herbal remedies in conventional medical treatments such as in flatulence, nausea, bronchitis, liver complaints, and anorexia. Besides, mint oil is used in the food industry as a food additive and taste enhancer

due to its olfactory features (Saric-Kundalic et al. 2009). This makes many *Mentha* species valuable for folk medicine and industry.

Several studies have confirmed that *Mentha* plants have plenty of phenolic acids (especially rosmarinic acid) and flavonoids (eriodictyol, luteolin, apigenin and their glycosides) (Brahmi et al. 2014). Moreover, essential oil (EO) as a complex and volatile organic compound with high biological activities could be extracted from all parts of mint vegetation. Generally, biological activities of mint species originate from their phenolics and essential oil components. In herbs, polyphenols ensure plants' protection against UV light, oxygen and nitrogen species, pathogens, and parasites (Elansary et al. 2016). Regarding EO, it is reported that the main component in *M. spicata* (spearmint) and *M. longifolia* (horsemint) is carvone (Kumar et al. 2015). Mahboubi (2018) found that carvone could cure flatulence and dysmenorrhea. Menthone, menthyl acetate, isomenthol, 1,8-cineole, and menthofuran are the other common major components in these two mints. Complex factors including genetic and environmental variations induce the chemical polymorphism in quantity and quality of essential oil composition (Crocoll et al. 2010). It is therefore inferred that crossing could create variability in mint essential oil components.

Over the years, hybridization has been considered as one of the most important techniques for plant breeders to combine desirable genes and to provide a new genetic resource aimed to select the superior genotypes in segregating generation (Scowcroft and Larkin 1982). Hybridization could lead to better performance, higher adaptability, and increase in quantity and quality of essential oil in the populations of mint (Khanuja et al. 2000). This requires the knowledge of genetic information on broad-sense heritability and genetic advance of interesting traits because it leads to increase the efficiency of selection in plant breeding (Guindon et al. 2019). In a study of intraspecific variation in four mints, Srivastava et al. (2003) declared that the value of h^2_b for oil yield, fresh weight, and dry weight were 98, 95, and 97%, respectively. Besides, Kumar et al. (2014) reported that the genetic advance for herb yield in half-sib seed progenies of *M. piperita* was 50.02.

After hybrid generation, transgressive segregation patterns in advanced generations play a crucial role in increasing genetic variation and to fix heterosis

(Ellstrand and Schierenbeck 2006; Kumar et al. 2018). Murray et al. (1972) crossed *M. spicata* and *M. aquatica* to generate 120,000 seedlings of mints. They found that “#57–1577-191” as a hybrid cross had the major essential oil constituents and strong “nasturtium” aroma rather than their parents. Tucker and Chambers (2002) crossed two clones of *M. longifolia*, (with a high amount of germacrene D and *trans*-piperitone oxide) with *M. arvensis* (with a high amount of 1, 8-cineole, pulegone, and linalool). They showed that most of the F_1 hybrids had higher essential oil constituents than the parents. Another example of transgressive segregation in *Mentha* was reported among 20 wild and cultivated clones of $M. \times gracilis$ and their 932 F_1 hybrids, which were evaluated by Tucker and Fairbrothers (1990) and Tucker et al. (1991). They showed that the hybrid “# 27–19” was known as one of the best clones in essential oil constituents and morphology. Reciprocal crosses concerning its cytoplasmic effects could be also considered as an appropriate strategy for breeding for hybrid vigor (Joshi et al. 2016). There is no information on reciprocal crosses in mint populations, including those of *M. spicata* and *M. longifolia*; however, Koseoglu et al. (2017) indicated that yield criteria of progenies in *Cicer arietinum* depend on the cytoplasmic effect of the female. They also revealed that the maternal effect is considered as the main factor to enhance seed sizes in progenies. Also, Bonea and Urechean (2003) found significant differences between direct and reciprocal crosses in maize referring to the cytoplasmic effect.

In the literature, there is little information on the variations of mint progenies derived from intraspecific crosses. The present study may be the first one aiming (1) to create the first and second generations of mint populations derived from intraspecific crosses within *M. spicata* and *M. longifolia* to estimate genetic parameters and genetic variation of the characters in order to select some mint chemotypes for promoting their medicinal properties, (2) to study the relative proportions of transgressive segregation and heterotic blocks through intraspecific hybridization to develop procedures for breeding these two *Mentha* species, (3) to describe the possible cytoplasmic effect in the study of direct and reciprocal crosses of horsemint populations and (4) to assess the amount of monoterpenes in the selected mints derived from intraspecific crosses in comparison with their parents. This may help breeders

to achieve a new genetic resource with a high level of secondary metabolites as well as the medicinal components, which could be used in the pharmaceutical industry.

Material and methods

Plant material

This study used two clones of Hamedan and Karaj from *M. longifolia* and two clones of Lorestan and Dehbala from *M. spicata* collected from various geographical areas of Iran (Heydarizadeh et al. 2013). These parental clones were selected based on their favorable characteristic (morpho-phenological traits and essential oil content) which was illustrated in the two previous articles (Heydarizadeh et al. 2013; Nazem et al. 2019). Besides, their crossability was proven in our previous paper (Yaghini et al. 2020).

In order to produce F_1 and F_2 generations, the genotypes of Karaj and Hamedan from *M. longifolia* were crossed together directly and reciprocally. The method of crossing has been illustrated previously (Yaghini et al. 2020). It should be noted that the direct cross (F_1) was performed when the Karaj clone was considered as the male parent, and the reciprocal cross (R- F_1) was done when the Hamedan clone was considered as the male parent. F_2 and R- F_2 were obtained from self-pollination of F_1 and R- F_1 , respectively. In the intraspecific crosses of *M. spicata*, due to the male sterility of Dehbala clone (Yaghini et al. 2020), it was crossed only as the female parent to the Lorestan clone as the male parent and F_2 generation was obtained from self-pollination of F_1 hybrids. In late February and early March 2018, seeds of F_2 generations were planted in a nursery. After several days, seedlings of F_2 generations along with F_1 plants and their parental clones were transferred into the experimental field on May 1st, 2018. Then, the best individuals of F_1 and F_2 from each cross were selected based on morphological and essential oil traits in order to evaluate the composition of their secondary metabolites.

Design of experiment

A RCBD (randomized complete block design) was applied to assess the parental clones, F_1 , and F_2 generations of both species at three replications in the

experimental field of Isfahan University of Technology. Each block with a size of 25 m (length) \times 10 m (width) consisted of twenty-five 1×10 m² plots (18 plots for the F_2 populations, 3 plots for the F_1 populations and 4 plots for the parents). Each plot contained 10 transplants from each parent or 10 seedlings from each F_1 or F_2 population with a distance of 100 cm between the plants. By this, 180 F_2 plants from each direct and reciprocal intraspecific cross of the two clones of *M. longifolia*, and 180 F_2 plants from the intraspecific cross of *M. spicata* were generated (in total, 540 plants from the F_2 s, 90 plants from F_1 generations and 120 plants from parents) and evaluated.

Morphological evaluation

The morphological study was carried out during the flowering season between August to September. Morphological data were recorded for each F_2 plant derived from intraspecific crosses along with their F_1 plants and parental clones. These included plant canopy diameter (cm), plant height (cm), fresh weight (g plant⁻¹), dry weight (g plant⁻¹), leaf area (mm²), the number of branches per plant, essential oil content, and essential oil yield.

Essential oil extraction

The essential oil was isolated using hydrodistillation of 100 g of shade-dried mint shoots as prescribed by European Pharmacopoeia (ver. 8.2, monograph 2.8.12) (Nazem et al. 2019). Then, the following equation was used to estimate the essential oil content (%) (Zhang et al. 2015):

$$\text{Essential oil (EO)(\%)} = \frac{\text{mass of EO (g)}}{\text{dried shoot (g)}} \times 100$$

GC-MS (Gas chromatography-mass spectrometry) analysis

Based on the data collected from morphological traits and essential oil yield, nine individuals along with their parents were selected in order to measure the essential oil components. The process of plant selection was based on a biplot analysis illustrated in the results and discussion section.

For an analysis of essential oil compositions, Agilent 7890 gas chromatograph (Agilent

Technologies, Palo Alto, CA, USA) along with an HP-5MS 5% capillary column and flame ionization detector (FID) was used. The column temperature was adjusted from 60 °C to 300 °C and held 3 min at 60 °C after that temperature raised 3 °C min⁻¹ to 120 °C gradually. Then, the temperature at a rate of 15 °C min⁻¹ increased from 60 °C to 300 °C and was subsequently remained at 300 °C for 5 min. The gas chromatographer was connected to an Agilent 5975 C (Agilent Technologies, Palo Alto, CA, USA) mass selective detector. The MS was armed with an ion-trap analyzer set at 1508 amu s⁻¹ with the voltage of 1350 of electron multiplier. Also, scanning at an ionization voltage of 70 eV in the 39–400 m/z range was accomplished.

Estimation of essential oil composition

The EO components of the selected individuals were determined by comparing their mass spectra with those attained by the NIST 08 (National Institute of Standards and Technology) based on the retention indices relative to C5-C24 n-alkanes obtained on a nonpolar HP-5MS column, Wiley 275.L (Adams 2005). The normalized peak area abundances were used for a semi-quantitative objective.

Assessment of total phenolic and flavonoid contents

Folin-Ciocalteu colorimetric method with minor modifications was used to determine total phenolic content of the selected plants (Pinelo et al. 2004). Briefly, in order to extract phenolics, 10 mL of methanol (80%) was supplemented to 250 mg of leaf powder, and then the extract was shaken at 25 °C with the application of a shaker at the speed of 150 rpm for eight h. Then, 2 mL of sodium carbonate solution (7.5%) was supplemented to 0.5 mL of extract diluted, and then it was mixed with 10% of Folin–Ciocalteu's reagent (2.5 mL). After, the mixture was incubated at 45 °C in a bath for 15 min. By using a spectrophotometer, the absorbance was recorded at 765 nm. Water and the reagents were used as a blank sample. The standard calibration curve was created by tannic acid (TAE) (0–500 ppm), and TPC was expressed as mg of TAE per gram of each extract on a dry basis (mg TAE/ g dry weight).

The aluminium chloride colorimetric method was used to determine the total flavonoid content (Pekal and Pyrzyńska 2014). First, 75 µL of 5% NaNO₂ was mixed with 125 µL of the extraction solution and maintained for 6 min. After adding 150 µL of AlCl₃ (10%), the solution was incubated for 5 min. Subsequently, 750 µL of NaOH (1 M) was supplemented to the solution and stored at room temperature for 15 min. Finally, the absorbance was measured at 510 nm in terms of mg of quercetin equivalents (QE) per gram of the extract.

Statistical analysis

Statistical analysis was performed by using ANOVA of the SAS statistical program according to a RCBD with three replications (version 9.1; SAS Institute Inc. Cary, NC, USA). LSD (least significant difference) test was employed to assess mean comparisons at a confidence level of 95%. PCA (principal component analysis) was also used to assess the relationships among all the measured traits and the mint populations. Statgraphics software was applied to draw a biplot in order to select the best progenies of each population.

Data used for analysis was the average of 10 plants from each parent, and F₁ hybrids and F_{2s} were evaluated individually.

In this study, heterosis (H) was determined using the following equation (Ene 2019):

$$H (\%) = \frac{MF_1 - MP}{MP} \times 100$$

In this equation, MF₁ is the mean value of F₁ population derived from intraspecific crosses, and MP is the mean value of parental clones.

Transgressive segregation index was also calculated by dividing the difference between the maximum and minimum of the traits in F₂ populations by the parental differences as follows (Koide et al. 2019):

$$\text{Transgressive Segregation Index} = \frac{\text{F}_2 \text{ difference}}{\text{Parental difference}}$$

Genetic parameters were determined for various characters through the variance components method (Larik et al. 1997) as follows:

Table 1 Analysis of variance (mean of squares) for the traits in F₁ and F₂ populations derived from the intraspecific cross of parental clones

Species	Source of Variation	df	Fresh weight	Dry weight	Number of branches plant ⁻¹	Plant height	Leaf area	Plant canopy diameter	Essential oil content	Essential oil yield
<i>M. longifolia</i>	Replication	2	331553 ^{ns}	39,323 ^{ns}	80.73 ^{ns}	316.5 ^{ns}	103,791 ^{ns}	337.2 ^{ns}	0.08 ^{ns}	0.11 ^{ns}
	Parental clones	1	951,908 ^{**}	95,435.1 ^{ns}	46.32 ^{ns}	1182.2 [*]	305,043 ^{**}	989.2 ^{**}	0.09 ^{ns}	0.07 ^{ns}
	F ₁ vs. R-F ₁	1	721,610 ^{**}	110,842.7 [*]	0.12 ^{ns}	15.71 ^{ns}	75,787 ^{ns}	434.3 [*]	0.59 ^{**}	32.6 ^{**}
	F ₂ vs. R-F ₂	1	10,507,851 ^{**}	1,045,734 ^{**}	2584 ^{**}	11,497 ^{**}	1,619,194 ^{**}	8431.3 ^{**}	0.21 ^{ns}	33.4 ^{**}
	F ₁ vs. F ₂	1	47329 ^{ns}	7137.3 ^{ns}	95.11 ^{**}	3253.6 [*]	817.3 ^{ns}	877.16 [*]	0.14 ^{ns}	4.9 [*]
	F ₁ vs. P	1	188704 ^{ns}	20,460 ^{ns}	20.19 ^{ns}	176.1 ^{ns}	4465 ^{ns}	128.3 ^{ns}	0.57 [*]	13.16 ^{**}
	F ₂ vs P	1	122352 ^{ns}	10,661 ^{ns}	98.32 ^{**}	1304.8 [*]	12,372 ^{ns}	1095.4 ^{**}	0.37 [*]	10.57 ^{**}
	Error	12	204,399	38,884.3	23.63	341.43	27,654	771.1	0.05	1
<i>M. spicata</i>	Replication	2	38,212.1 ^{ns}	7585 ^{ns}	10.5 ^{ns}	644.7 ^{ns}	25,221 ^{ns}	803.2 ^{ns}	0.01 ^{ns}	0.07 ^{ns}
	Parental clones	1	52,174.4 ^{ns}	9730 ^{ns}	60.3 ^{ns}	2120 ^{**}	596,796 ^{**}	3740.5 [*]	0.01 ^{ns}	0.04 ^{ns}
	F ₁ vs. F ₂	1	141,810.4 ^{ns}	117,442 ^{**}	1074.9 ^{**}	1634.7 [*]	68,913 ^{ns}	8675.5 ^{**}	0.12 [*]	10.01 ^{**}
	F ₁ vs. P	1	1,165,174 ^{**}	222,882 ^{**}	1110.4 ^{**}	6528.4 ^{**}	771,312 ^{**}	17,399.5 ^{**}	2.6 ^{**}	21.4 ^{**}
	F ₂ vs P	1	924,555 [*]	51,952 [*]	38.86 ^{ns}	3503.1 ^{**}	721,575 ^{**}	4313.5 [*]	0.81 ^{**}	18.1 ^{**}
	Error	6	84,651	6966	50.62	136.04	28,870	577.7	0.049	0.8

*and**, significant at $P < 0.05$ and $P < 0.01$, respectively and ns: non-significant

GCV% (Genotypic Coefficient of Variability)

$$= \frac{\sigma_g}{MF_2} \times 100$$

PCV% (Phenotypic Coefficient of Variability)

$$= \frac{\sigma_p}{MF_2} \times 100$$

MF₂: mean value of the F₂ generation.

Broad – sense heritability (h_b^2) = $\frac{\sigma^2_g}{\sigma^2_p}$

Genetic advance (GA) = $i \times h_b^2 \times \sigma_p$, where $i = 2.06$ at 5% selection intensity (Kang et al. 1983). σ^2_g and σ^2_p are the genotypic and phenotypic variances, respectively (Abebe et al. 2017).

Results and discussion

Morphological analysis

Results showed significant differences ($p \leq 0.01$) for most morphological traits among the parental clones, populations derived from intraspecific crosses, and

parental clones versus populations derived from intraspecific crosses in both species (Table 1). The mean values of morphological characteristics and secondary metabolites in mint plants are summarized in Table 2.

Fresh and dry weights

Overall among all parental clones in both species, Karaj as the parental clone from *M. longifolia* had the highest fresh weight (564.65 g per plant) and dry weight (205.13 g per plant), while the lowest fresh weight (247.16 g per plant) and dry weight (94.18 g per plant) were observed in Lorestan as the parental clone from *M. spicata* (Table 2). Table 1 indicates significant differences between the F₁ population derived from the intraspecific cross of *M. longifolia* versus the R-F₁ population. The higher average of fresh weight in R-F₁ (579.68 g per plant) rather than that of F₁ (389.08 g per plant), indicates that cytoplasmic genes are probably playing a role and affecting the hybrid vigor (Joshi et al. 2016). In the F₂ population of *M. longifolia*, the fresh weight ranged from 111 to 2150.23 g per plant, and dry weight varied

Table 2 Mean comparisons for different traits among mint clones, F₂ and F₁ generations derived from intraspecific crosses

Species	Populations	Fresh weight (g plant ⁻¹)	Dry weight (g plant ⁻¹)	Number of branches plant ⁻¹	Plant height (cm)	Leaf area (mm ²)	Plant canopy diameter (cm)	Essential oil content (%)	Essential oil yield (g plant ⁻¹)
<i>M. longifolia</i>	Hammedan	343.83 ± 14.8 ^{bc}	140.05 ± 11.8 ^{ab}	10 ± 0.2 ^b	54.91 ± 2.2 ^{ab}	211.01 ± 11.7 ^c	68.57 ± 3.8 ^b	1.13 ± 0.07 ^c	1.58 ± 0.2 ^c
	Karaj	564.65 ± 17.2 ^a	205.13 ± 14.7 ^a	12 ± 0.1 ^b	65.05 ± 4.3 ^a	360.79 ± 13.2 ^a	77.84 ± 4.3 ^a	0.76 ± 0.05 ^d	1.55 ± 0.2 ^c
	F ₁	389.08 ± 18.7 ^{bc}	157.83 ± 10.3 ^{ab}	12 ± 0.5 ^b	62.1 ± 4.2 ^a	248.9 ± 9.3 ^{bc}	74.99 ± 6.7 ^a	1.12 ± 0.1 ^c	1.76 ± 0.8 ^c
	R- F ₁	579.86 ± 13.2 ^a	237.55 ± 13.2 ^a	12 ± 0.3 ^b	63.17 ± 4.7 ^a	323.2 ± 12.9 ^{ab}	67.29 ± 5.2 ^b	1.68 ± 0.1 ^a	3.9 ± 1.1 ^{2a}
	F ₂	281.89 ± 31.3 ^c	129.36 ± 29.2 ^b	11 ± 1.7 ^b	48.28 ± 5.2 ^b	210.8 ± 24.2 ^c	60.89 ± 9.4 ^b	1.23 ± 0.2 ^{bc}	1.59 ± 0.69 ^c
	R-F ₂	645.2 ± 39.2 ^a	243.94 ± 30.7 ^a	17 ± 1.1 ^a	60.29 ± 5.1 ^a	352.87 ± 28.7 ^a	71.18 ± 9.2 ^a	1.48 ± 0.18 ^b	3.61 ± 0.98 ^b
<i>M. spicata</i>	Lorestan	247.16 ± 11.2 ^c	94.18 ± 19.8 ^c	10 ± 0.5 ^b	25 ± 1.61 ^c	130.63 ± 7.2 ^b	37.8 ± 2.2 ^c	0.91 ± 0.09 ^b	0.85 ± 0.2 ^c
	Dehbala	322.13 ± 13.1 ^{bc}	126.56 ± 11.3 ^{bc}	13 ± 0.7 ^b	39.06 ± 2.24 ^b	363.1 ± 9.3 ^a	56.47 ± 1.4 ^b	0.7 ± 0.1 ^b	0.88 ± 0.1 ^c
	F ₁	549.63 ± 33.3 ^a	206.26 ± 18.2 ^a	20 ± 0.3 ^a	51.26 ± 2.04 ^a	454.65 ± 17.8 ^a	78.53 ± 1.3 ^a	1.3 ± 0.3 ^a	2.67 ± 0.48 ^a
	F ₂	470.76 ± 44.2 ^{ab}	144.48 ± 42.7 ^b	13 ± 1.1 ^b	42.84 ± 1.5 ^b	400.07 ± 24.3 ^a	59.13 ± 4.2 ^b	1.53 ± 0.4 ^a	2.21 ± 0.7 ^b

In each column in any species, means followed by the same letter are not significantly different at $P < 0.05$ probability level

from 67 to 720.3 g per plant (data not shown). Nazem et al. (2019) reported that the range of fresh weight changed from 474.8 to 1290.08 g per plant, while dry weight ranged from 153.22 to 411 g per plant in *M. longifolia*. The transgressive segregation index of F₂ progenies of *M. longifolia* was 19.3 (Table 3), indicating that the fresh weight of many plants in the F₂ population exceeded those of their parents. Transgressive segregation in F₂ and F₃ generations for yield and yield criteria has been previously observed in reciprocal interspecific crosses between *C. reticulatum* Ladiz and *Cicer arietinum* L. (Koseoglu et al. 2017).

The mean of fresh and dry weights in the F₁ population of *M. spicata* was 549.63 and 206.26 g per plant, respectively. In the F₂ generation from the cross of *M. spicata*, the mean of fresh and dry weights was 470.76 and 144.48 g per plant, respectively (Table 2). The higher mean of fresh and dry weights in the F₁ population rather than those in the F₂ population is probably due to the presence of dominant alleles and heterosis phenomena (Ene et al. 2019). According to the estimation of genetic parameters, the percentage of heterosis for fresh and dry weights in intraspecific crosses of *M. spicata* was 93 and 87%, respectively (Table 3).

Number of branches plant⁻¹

The number of branches per plant in parental clones from *M. longifolia* (12 and 10 branches per plant) and *M. spicata* (13 and 10 branches per plant) had a narrow range. This experiment showed that the progenies of F₁ and R-F₁ with the same branches per plant did not have a significant difference versus their parental clones (Table 2). In contrast, the F₁ population derived from the intraspecific cross of *M. spicata* had a higher number of branches per plant (20) compared with the parental clones (12 branches per plant) and F₂ population (13 branches per plant). By this, the heterosis obtained from the F₁ cross belonging to *M. spicata* was 73%. The largest number of branches per plant in the intraspecific cross of *M. longifolia* was related to the R-F₂ population (17 branches per plant). This was led to the higher values of genetic advance (34) and transgressive segregation index (18.5) in the R-F₂ population of this species (Table 3).

Table 3 Estimates of genetic parameters in the progenies of mint derived from intraspecific crosses

Species	Characters	Fresh weight	Dry weight	Number of branches plant ⁻¹	Plant height	Leaf area	Plant canopy diameter	Essential oil content (%)	Essential oil yield
<i>M. longifolia</i> (direct cross)	Genetic Coefficient of Variability (GCV %)	41.4	37.2	10.8	12.4	24.4	14.6	30	53.4
	Phenotype Coefficient of Variability (PCV %)	62.2	59	37.2	23.2	43	26.9	34.9	106.2
	Heritability % (h^2_b)	71	57	58	23	29	18	72	71
	Genetic advance in F ₂ population (5%)	83	26.2	4.2	8.89	37.3	10.9	99	90.3
	Heterosis %	- 12	- 8	9	3	- 10	2	18	12
	Percentage of the F ₂ plants transgressed	45	40	52	55	48	45	76	83
	Transgressive segregation index	19.32	11.04	10	6.4	5.9	8.3	3.46	120.3
	Genetic Coefficient of Variability (GCV %)	37.6	38.1	19.8	1.32	20.6	9.4	29.7	54.8
	Phenotype Coefficient of Variability (PCV %)	51.2	48.4	34.7	23.6	32.7	22	25.6	94.7
	Heritability % (h^2_b)	78	43	34	19	52	18	72	73
<i>M. spicata</i>	Genetic advance in F ₂ population (5%)	330.6	106.9	34	0.16	96	5.95	79	89.3
	Heterosis %	41	37	9	5	13	- 8	78	154
	Percentage of the F ₂ plants transgressed	38	43	61	34	29	35	95	90
	Transgressive segregation index	17.5	10.3	18.5	6.7	9.3	8.5	3.3	70
	Genetic Coefficient of Variability (GCV %)	47.2	49.7	35.3	17.1	35.1	21.9	20.2	52.6
	Phenotype Coefficient of Variability (PCV %)	70.1	81.8	45.3	16.8	28.7	26.5	24.8	154
	Heritability % (h^2_b)	72	57	64	25	62	22	76	89
	Genetic advance in F ₂ population (5%)	362.1	112.7	6.33	17.6	223.3	22.8	78.3	80.9
	Heterosis %	93	87	73	60	84	66	62	210
	Percentage of the F ₂ plants transgressed	56	51	54	40	35	40	69	73
Transgressive segregation index	28.2	7.2	16.5	5.5	12.9	9.3	9	16.25	

Plant height

Based on the results (Table 1), significant differences were obtained among the parental clones that belonged to *M. longifolia* for plant height. The mean value of plant height in Hamedan and Karaj was 54.91 cm and 65.05 cm, respectively. Significant differences were also observed between the F₁ and F₂ populations derived from intraspecific crosses of *M. longifolia* (Table 1). This means that selection in advanced generations may increase the plant height in this species. Transgressive segregation was also present in this species because the lowest and the highest plant height in the progenies were 31 cm and 99 cm belonged to F₂ and R-F₂ populations (data not shown). The mean value of plant height in Lorestan and Dehbalá from *M. spicata* was 25 cm and 40 cm, respectively. Although in the F₁ generation, the mean value of plant height (51.26 cm) increased due to the heterosis (60%), the mean value of this trait in the F₂ population of *M. spicata* decreased to 42.84 cm. Smolik et al. (2007) reported that the average height of *M. longifolia* and *M. spicata* amounted to 56.05 cm and 43.16 cm, respectively.

Leaf area

Statistical analysis showed significant differences in terms of leaf area among the parental clones belonged to both species (Table 1). Leaf area in Dehbalá and Lorestan belonged to *M. spicata* was 130.63 mm² and 363.1 mm², respectively. The first generation derived from the intraspecific cross of *M. spicata* with 454.65 mm² had the highest value of leaf area either relative to the parents or relative to the F₂ generation (Table 2). Heterosis (84%) and accumulated fixable genes may play essential roles in the expressed high value of leaf area in F₁ generation (Ene et al. 2019). The results support the findings of Golabadi et al. (2015), who also observed heterosis in leaf length and width in intraspecific crosses of cucumber. Although the mean leaf area in the second generation of the intraspecific cross of *M. spicata* was 400.07 mm², less than that of the first generation, the highest leaf area in the F₂ population with 1035.6 mm² (data not shown) represented transgressive segregation. In *M. longifolia*, the mean leaf area of F₁ (248.9 mm²), R-F₁ (323.2 mm²), F₂ (210.8 mm²), and R-F₂ (352.87 mm²) were all placed in the range of Hamedan (211.01 mm²) and

Karaj (360.79 mm²) as the parental clones. Nazem et al. (2019) in the study of self-and open-pollinated populations of mint reported that the highest and lowest leaf area per leaf were 1404.68 mm² and 423.84 mm², respectively.

Plant canopy diameter

In this study, the diameter of a plant canopy in parental clones ranged from 37.8 cm in Lorestan from *M. spicata* to 77.84 cm in Karaj from *M. longifolia* (Table 2). Heterosis was a dominant phenomenon in the intraspecific cross of *M. spicata*, so that the diameter of the plant canopy in the F₁ generation with 78.53 cm was higher than that in the parental clones. The heterosis level in this cross was estimated to be 66%. In the F₂ generation, the diameter of the plant canopy reached 59.13 cm; the reduction of this trait in the F₂ relative to F₁ generation can be attributed to inbreeding depression. In contrast, the diameter of plants canopy in *M. longifolia* reached 71.18 cm in the R-F₂ population compared with the F₂ population with 60.89 cm. This indicated that the plant's canopy diameter of progenies might depend on the female in this species, which may be considered as the cytoplasmic effect of the female as the main reason. Although there is no study on the value of heterosis in intraspecific crosses of mint, Koseoglu et al. (2017) showed that the average heterosis for all traits in direct interspecific crosses between *C. reticulatum* and *C. arietinum* was higher than that of its reciprocal crosses.

Chemical analysis

Essential oil content

Based on the results, a significant difference ($p \leq 0.01$) for the essential oil content was observed between the F₂ population versus parental clones (1.53 vs. 0.8) and the F₁ population versus parental clones (1.3 vs. 0.8) in *M. spicata* (Table 1). The essential oil content of the F₂ generation was also higher than that of F₁ and parental clones. It means that increasing essential oil content in this species may be affected by transgressive segregation. In other words, the complementary action of additive alleles that are accumulated in the F₂ generation is probably the cause of this increase. In contrast, in populations belonging to *M.*

longifolia, the essential oil content of R-F₁ (1.68) was higher than that of F₁ (1.12), F₂ (1.23), R-F₂ (1.48) and parental clones (0.94). It is probably due to both the expression of heterosis and cytoplasmic effects. Results may show that genetic recombination after hybridization, increased the essential oil content of mint due to the improvement in the genetic background. Some other factors such as ploidy level, in vitro-induced polyploidy, plant phenological stage, and pollination system, may lead to a change in the content of essential oil in pharmaceutical plants (Meier and Mediavilla 1998; Talebi et al. 2016).

Populations showed different responses to direct and reciprocal crossings in *M. longifolia*. It is expected that cytoplasmic effects play a major role in the essential oil production between the mint species. The difference between direct and reciprocal crosses may also refer to the inheritance of maternally supplied episomes such as mitochondrial DNA, the inheritance of uni-parental epigenetic markers, and inheritance of sex chromosomes (Brenner and Miller 2001). In contrast to our study, Gyenesne Hegyi et al. (2001), in the study of protein and oil contents of 12 direct and 12 reciprocal hybrids, found no statistical difference in the oil content between direct and reciprocal hybrids of maize.

Essential oil yield

According to the analysis of variance, significant differences ($p \leq 0.01$) were observed among the populations in both species (Table 1). In *M. spicata*, the essential oil yield of Lorestan and Dehbala as the parental clones with the value of 0.85 and 0.88 g per plant, respectively, were lower than the essential oil yield of the F₁ population with 2.67 g per plant and F₂ population with 2.21 g per plant. Crossing in this species may be considered as a factor for increasing the amount of essential oil yield. In *M. longifolia*, essential oil yield in the F₂ population was 1.59 g per plant, while in the R-F₂ population it was 3.61 g per plant. The same trend was observed in the first generation of crossing in *M. longifolia* as in F₁ and R-F₁ populations, the essential oil yield was 1.76 and 3.9 g per plant, respectively (Table 2). It seems that the reciprocal cross is contributed to increase essential oil yield, which could help breeders to improve the efficacy of future breeding programs. Former studies have also shown that the value of essential oil yield

ranged from 0.77 to 4.99 g per plant and 2.49 to 10.61 g per plant in *M. spicata* and *M. longifolia*, respectively (Nazem et al. 2019). Various ecological factors, including relative humidity, temperature, photoperiod, and irradiance, impress the EO yield of *Mentha* spp. (Fahlen et al. 1997). In our study, it is evident that genetic factors like direct and reciprocal crosses are also contributing to the trait values.

Transgressive segregations and the process of plant selection

The transgressive segregation index of the F₂ population derived from the intraspecific cross of *M. longifolia* and *M. spicata* indicated that essential oil yield, dry weight, and fresh weight of many plants exceeded the range of their parents (Table 3). There is some evidence for transgressive segregation in the crosses of other species. Koide et al. (2019) reported a strong transgressive index (25.7) for days to heading in rice crosses. Koseoglu et al. (2017) also showed that the range in most of the traits in F₂ and F₃ were higher than the range of parents in cicer crosses. The presence of transgressive phenotypes in segregating populations may be related to the high mutation rate in hybrids, epistasis, overdominance, revealing of rare recessive alleles that are typically heterozygous in parental taxa, variation in chromosome numbers, and the complementary action of additive alleles that are diffused between parental lines (Rieseberg et al. 1999). Therefore, by using intraspecific hybridization in *Mentha*, we could improve the plant's yield and yield criteria via the generation of new genetic resources.

Based on morphological traits and essential oil percentage and yield, some superior plants of F₁ and F₂ generations, along with their parents, were selected and their essential oil composition and secondary metabolites were determined. This was done according to Fig S. A, Fig S. B, and Fig S. C.

Essential oil composition

Sixteen and twenty-five oil compounds were identified in *M. longifolia* and *M. spicata*, respectively (Table 4). The sum of the percentages of recognized compounds varied from 90.42 to 97.02%. The main compounds in the essential oil of *M. longifolia* were pulegone, menthon, and piperitenone. These findings on

Table 4 Volatile compounds (percentage) of essential oils in parental clones, and selected F₁ and F₂ genotypes of *M. longifolia* and *M. spicata*

Compositions (%)	RI	<i>M. longifolia</i>										<i>M. spicata</i>				
		Karaj	Hamedan	399 (F ₁)	197 (R-F ₁)	305 (F ₂)	227 (F ₂)	33 (R-F ₂)	34 (R-F ₂)	Lorestan	Dehbala	134 (F ₁)	181 (F ₂)	77.69	92.63	
Monoterpene	—	88.91	90.27	89.3	91.94	89.22	88.92	92.38	91.01	85.5	74.65	80.12	77.69	92.63		
α-Pinene	939	0.9	0.84	0.74	1.03	0.9	0.65	0.75	0.67	0.78	1.22	0.34	0.51	2.27		
Sabinene	973	0.73	0.79	0.83	0.99	0.55	0.69	0.98	0.56	1.49	1.44	0	0.65	0.63		
β-Pinene	976	0.84	0.61	0.61	0.8	0.8	0.85	0.75	1.02	0.001	0.003	0.93	0.78	0.47		
β-Myrcene	989	0.001	0.002	0	0.09	0.58	0.84	0.38	0.29	1.04	1.86	0	0.97	0.35		
Limonene	1029	8.95	4.33	13.64	3.70	0.55	4.27	4.21	6.79	11.56	13.76	23.27	9.67	19.96		
1,8-Cineole	1031	0.72	0.08	0.81	0	1.7	1.12	1.5	0.39	0.01	0.1	0	1.89	1.71		
Ocimene	1037	0	0	0	0	0	0	0	0	0.01	0.02	0	0.8	0		
Linalool	1099	0	0	0	0	0	0	0	0	1.2	1.42	0	0.44	0.84		
Menthone	1150	28.02	26.6	0.61	39.36	52.8	3.62	1	0.02	0.001	0.005	0	0	0.34		
Isopulegone	1176	1.5	1.27	0	1.21	0.85	0	0.97	1.4	0.03	0.001	0	0	0.5		
Borneol	1166	0	0	0	0	0	0	0	0	0.65	0.96	11.19	0.4	0.4		
Neodihydrocarveol	1194	0	0	0	0	0	0	0	0	0.65	2.07	4.32	1	7.56		
Dihydrocarvone	1201	0	0	0	0	0	0	0	0	7.52	4	12.68	12.5	2.1		
Trans-Carveol	1217	0	0	0	0	0	0	0	0	7.04	9.98	1.29	4.89	0		
Cis-Carveol	1226	0	0	0	0	0	0	0	0	30	31	0	2.3	22		
Pulegone	1234	24.94	41.92	8.17	33.36	16.89	1.3	10.76	4	0.01	0.1	11.19	0.88	3.3		
Carvone	1242	0.1	0.01	1.55	0	0	0.01	0	0	23.41	6.52	14.91	38.27	27.01		
Piperitone	1253	3.6	2.2	0	4.7	3.1	4	3.4	3	0	0	0	0	0		
Piperitone oxide	1254	2.8	3.2	0	1.7	0	0	2	2.2	0	0	0	0	0		
Piperitenone	1345	15.8	8.32	58.41	5	10.5	67.76	57.67	61.5	0.1	0.2	0	1.74	3.19		
Piperitenone oxide	1363	0.01	0.1	3.93	0	0	3.81	8.01	9.17	0	0	0	0	0		
Sesquiterpene	—	1.92	4.551	1.12	1.27	1.75	1.93	2.75	3.71	4.46	3.88	7.01	10.52	2.23		
Beta-bourbonene	1384	0	0	0	0	0	0	0	0	0.67	0.75	0	1.5	0.11		
Beta-cubebene	1386	0	0	0	0	0	0	0	0	1.08	0.63	6.3	8.42	0.4		
Caryophyllene	1420	1.91	4.55	1.12	1.27	1.26	1.6	2.75	3.71	1.4	1.2	0.71	0	1.22		
Alpha-humulene	1453	0	0	0	0	0	0	0	0	0.01	0.002	0	0	0.3		
Germacrene D	1480	0.01	0.001	0	0	0.49	0.33	0	0	1.3	1.3	0	0.6	0.2		
Others	—	0	0	0	0	0	0	0	0	7.06	13.97	5.4	6.51	0.9		
Endo-bornyl acetate	1283	0	0	0	0	0	0	0	0	0.01	0.02	0	0	0.1		
Dihydrocarvyl acetate	1336	0	0	0	0	0	0	0	0	7.05	13.95	5.4	6.51	0.8		

Table 4 continued

Compositions (%)	<i>M. longifolia</i>					<i>M. spicata</i>							
	Karaj	Hamedan	399 (F ₁)	197 (R-F ₁)	305 (F ₂)	227 (F ₂)	33 (R-F ₂)	34 (R-F ₂)	Lorestan	Dehbala	134 (F ₁)	181 (F ₂)	95 (F ₂)
Total	90.83	94.82	90.42	93.21	90.97	90.85	95.13	94.72	97.02	92.51	92.53	94.72	95.76

RI: Retention indices on the HP-5MS column, Karaj: Parental clone of *M. longifolia*, Hamedan: Parental clone of *M. longifolia*, #399 (selected genotype of F₁ generation), #197 (selected genotype of R-F₁ generation), #227 and #305 (selected genotypes of F₂ generation) and #33 and #34 (selected genotypes of R-F₂ generation). Lorestan: Parental clone of *M. spicata*, Dehbala: Parental clone of *M. spicata*, #134 (selected genotype of F₁ generation); #95 and #181 (selected genotypes of F₂ generation)

major essential oil compositions of *M. longifolia* were in agreement with the results of Diop et al. (2016) and Yahia et al. (2018). The amount of these compositions changed between the populations belonging to these species. For instance, the percentage of pulegone in the EO of *M. longifolia* was from 1.3% in genotype #227 belonged to the F₂ generation to 41.92% in the parental clone of Hamedan. Therefore, the quantity of pulegone decreased in the selected plants of the intraspecific cross. Although the percentage of piperitenone in Karaj and Hamedan was 15.8 and 8.32%, respectively, the amount of piperitenone in the selected population derived from the intraspecific cross (67.76%) placed out of the range of their parents. Similar to piperitenone, the percentage of menthone in the selected population derived from the intraspecific cross (52.8%) placed out of the parental clones' range. The maximum percentages of piperitenone and menthone belonged to genotypes #227 and #305, respectively; both related to the F₂ generation of *M. longifolia* crosses. Therefore, the intraspecific cross could increase the value of these compounds in the F₂ generation of this species probably due to the presence of transgressive segregation.

Carvone and limonene were the major compositions in the EO of *M. spicata* in the previous study (Nazem et al. 2019). The percentages of limonene in the Lorestan clone (11.56%) was lower than that in the Dehbala clone (13.76%). However, in the F₁ population, the amount of limonene raised to 23.27% possibly due to the heterotic effect, but in selected F₂ population, the percentage of this compound reduced to 9.67% in the genotype #181 and 19.96% in the genotype #95 (Table 4). In contrast, the amount of carvone in the Lorestan clone (23.41%) was higher than that in the Dehbala clone (6.52%). The highest percentages of carvone were 38.27 and 27.01% belonged to the genotypes #181 and #95 related to the F₂ population derived from the intraspecific cross of *M. spicata* (Table 4). It seems that intraspecific cross in *M. spicata* could increase the percentage of these compounds.

The results of this study clearly show that selection based on the phenotypic traits in both species could increase the volume of specific essential oil compositions such as limonene, menthone, carvone, and piperitenone in F₁ and F₂ generations. Classifying the terpenoids in Table 4 shows that the volume of monoterpenes and sesquiterpenes did not change

significantly in the offspring of intraspecific crosses of *M. longifolia* in comparison with the superior parent. In *M. spicata*, monoterpenes in Lorestan and Dehbala included 85.5 and 74.65% of the oil, respectively. The amount of monoterpenes in F₁ generation was in the parental range, but monoterpenes level in the genotype #95 of the F₂ generation increased and reached 92.63% (Table 4). Also, the genotype #181 belonging to the F₂ population of *M. spicata* had a significantly higher content of sesquiterpenes than that of both parents and reached 10.52% (Table 4). It seems that selfing in *M. spicata* could increase the amount of monoterpenes and sesquiterpenes in F₂ generations. The results of Nazem et al. (2019) was in line with our results. They also showed that selfing in *M. spicata* as a genetic mechanism increased the amount of these groups of components. Environmental factors such as altitude, relative humidity, temperature, rainfall, and plant's phenological stages may also induce these variations in the main compounds of EOs (Riahi et al. 2013).

TPC (total phenolic content) and TFC (total flavonoid content)

The results showed significant differences ($p \leq 0.05$) in TPC values between parental clones in *M. spicata* and *M. longifolia*, (Figs. 1, 2, 3). The lowest TPC value among parental clones was related to Hamedan with 7.46 mg TAE g⁻¹ DW. The maximum value of TPC in the intraspecific cross of *M. longifolia* belonged to the genotype #114 of the R-F₂ population with 13.88 mg TAE g⁻¹ DW (Fig. 2). It showed that selection based on morphological and essential oil traits could improve the value of TPC in the R-F₂ generation. Abootalebian et al. (2016) reported that the highest value of TPC in *M. longifolia* was 67.2 mg TAE g⁻¹ DW which is significantly higher than the content observed in our study. Different factors such as genetic structure and phenological stage of harvested plants and the extraction method employed should be at the origin of these variations in the phenolic content of medicinal plants (Gharibi et al. 2015). In contrast to *M. longifolia*, in the intraspecific cross of *M. spicata*, the highest value of TPC with 27.84 mg TAE g⁻¹ DW belonged to Lorestan as the parental clone (Fig. 3). Therefore, intraspecific cross in this species probably could not increase the value of TPC in favor of their progeny.

All of the parental clones belonging to both species exhibited significant differences ($p \leq 0.05$) in terms of total flavonoid content values. TFC in parental clones ranged from 4.35 mg QE g⁻¹ DW in Lorestan to 10.19 mg QE g⁻¹ DW in Karaj. The highest amount of TFC in the intraspecific cross of *M. spicata* genotypes belonged to the genotype #96 from the F₂ population with 9.67 mg QE g⁻¹ DW (Fig. 3), which may show the presence of transgressive segregation. The maximum value of TFC in the intraspecific cross of *M. longifolia* was related to the genotype #114 as an R-F₁ plant with 11.85 mg QE g⁻¹ DW (Fig. 2). This increase in TFC may be due to the effect of heterosis and/or cytoplasmic effect. In *M. longifolia*, the mean of TFC in R-F₁ (8.44 mg QE g⁻¹ DW) was significantly higher than that of the F₁ population (6.41 mg QE g⁻¹ DW). The same trend was observed in the F₂ generation. It means that the R-F₂ population derived from the intraspecific cross with 7.55 mg QE g⁻¹ DW has a significantly higher amount of phenolics and flavonoids in comparison with the F₂ population (5.99 mg QE g⁻¹ DW). Overall, it is likely that reciprocal crossing in this species has increased the value of TFC in comparison with the direct crossing.

From a metabolic point of view, there were significant differences ($p \leq 0.01$) between the mean of F₂ population (8.07 mg QE g⁻¹ DW) versus parental clone (5.16 mg QE g⁻¹ DW) in populations belonging to *M. spicata*. In contrast, the mean of TFC in the F₁ population (5.4 mg QE g⁻¹ DW) from *M. spicata* placed in the range of parental clones. This showed that F₁ hybrids probably included combining genes from both parents. Prior studies have also indicated that the TFC value of *Mentha* spp. ranged from 2.34 to 20.35 mg QE g⁻¹ DW (Nazem et al. 2019). These results also confirmed that the selection based on morphological traits could help breeders to achieve a genotype with a higher amount of TPC and TFC.

Heritability, coefficient of variation and genetic advances

Genetic parameters computed through the intraspecific cross of mint clones demonstrated a high to moderate heritability and genetic advance for almost all morphological characters except for plant height and plant canopy diameter (Table 3). Essential oil yield, essential oil content, and fresh weight showed

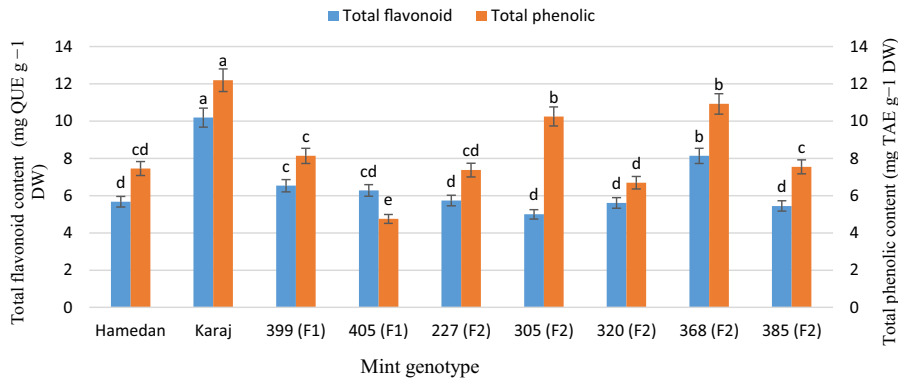


Fig. 1 The value of total phenolic and total flavonoid contents among parental clones and F₁ and F₂ generations derived from the direct intraspecific cross of *M. longifolia*. Hamedan: Parental

clone, Karaj: Parental clone, #399 and #405 (selected genotypes of the F₁ generation): #227, #305, #320, #368 and #385 (selected genotypes of the F₂ generation)

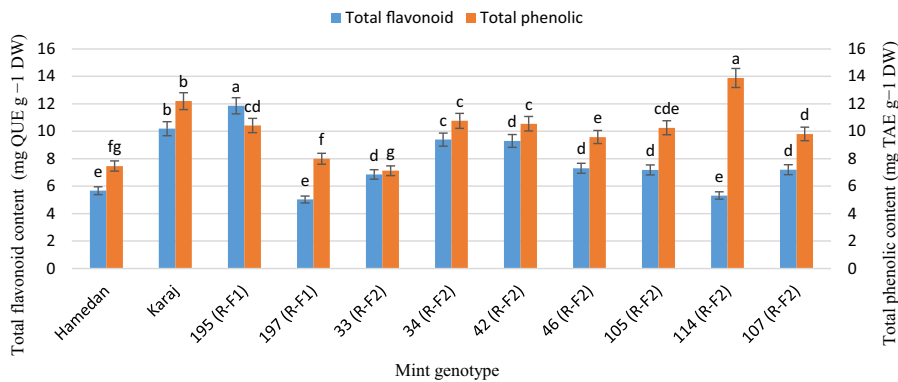


Fig. 2 The value of total phenolic and total flavonoid contents among parental clones and F₁ and F₂ generations derived from the reciprocal intraspecific cross of *M. longifolia*. Hamedan:

Parental clone, Karaj: Parental clone, #195 and #197 (selected genotypes of the R-F₁ generation): #33, #34, #42, #46, #105, #114 and #107 (selected genotypes of the R-F₂ generation)

high values of heritability (> 70%) in both intraspecific crosses (Table 1). Srivastava et al. (2003) found similar results in *M. arvensis* and reported high broad-sense heritability for oil yield, fresh and dry weights.

According to Manju and Sreelathakumary (2002), high narrow-sense heritability with a large number of fixable additive factors allow selection to improve desired traits. Kumar et al. (2003) and Kumar and Mishra (2004) proved that selecting the best lines of wheat at first segregating generation could be beneficial for traits with high genetic advances and high heritability. Although Kumar et al. (2014) in the study of eight half-sib seed progenies of *M. piperita* showed a high heritability, along with high genetic advances for plant height, in our study, low estimates of broad-sense heritability accompanied with a low genetic

advance in the F₂ generation for plant height and plant canopy diameter in both intraspecific crosses suggested that these characters are poorly heritable and may be changed due to non-additive gene action and presence of G × E interaction.

According to the results of Table 3, a high level of genotypic coefficients of variation was observed for fresh weight, dry weight, and essential oil yield in both species. A moderate level of genotypic coefficient of variation was seen in the case of leaf area, and a low level of genotypic coefficient of variability was seen in the case of plant height. In terms of phenotypic coefficient of variation, the same trend was observed in both species. For example, PCV ranged from 154 percent for essential oil yield to 16.8 percent for plant height; both of which are related to

Fig. 3 The value of total phenolic and total flavonoid contents among parental clones and F₁ and F₂ generations derived from the intraspecific cross of *M. spicata*. Lorestan: Parental clone, Dehbala: Parental clone, #133 and #134 (selected genotypes of the F₁ generation): #95, #96, #181 and #104 (selected genotypes of the F₂ generation)

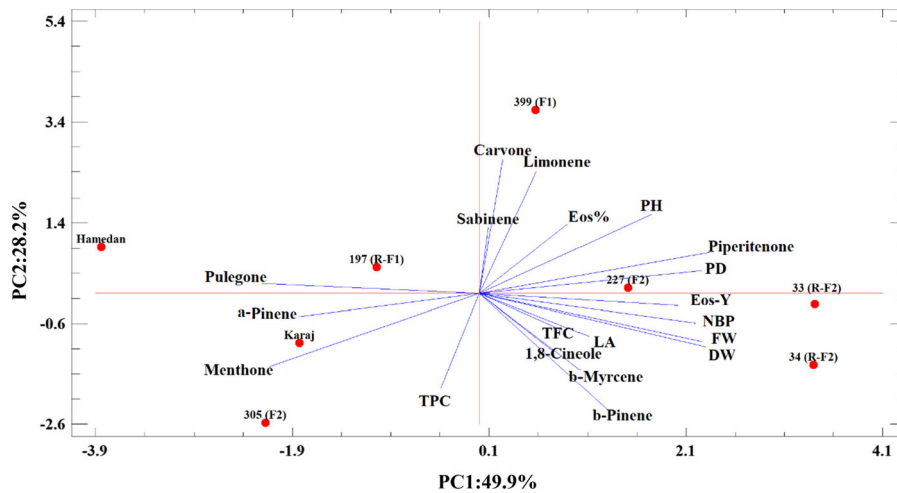
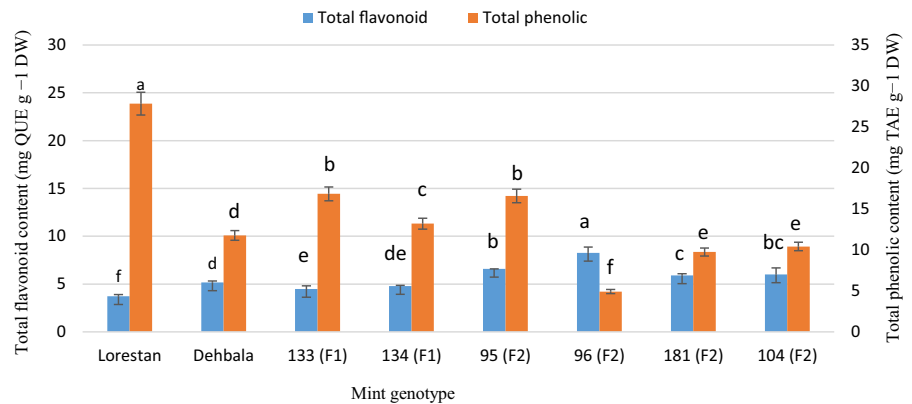


Fig. 4 PCA analysis for the classification of selected F₁ and F₂ mint genotypes along with their parents based on growth traits and secondary metabolites in *M. longifolia*. Hamedan: Parental clone, Karaj: Parental clone, #399 (selected genotype of the F₁ generation): #227 and #305 (selected genotypes of the F₂ generation), #197 (selected genotype of the R-F₁ generation):

#33 and #34 (selected genotypes of the R-F₂ generation). PH: Plant height, NBP: Number of branches plant⁻¹, PD: Plant canopy diameter, FW: Fresh weight, DW: Dry weight, LA: Leaf area, Eos %: Essential oil content, Eos-Y: Essential oil yield, TPC: Total phenolic content, TFC: Total flavonoid content, α -Pinene: α -pinene, β -Myrcene: β -Myrcene, β -Pinene: β -pinene

the intraspecific cross of *M. spicata*. The highest percentage of GCV offers an opportunity for better selection and augmentation for fresh weight and essential oil yield in the generations of intraspecific crosses of mint. Burton and de Vane (1953) stated that GCV coupled with heritability estimates the genetic enhancement through phenotypic selection. In general, the higher percentage of PCV rather than GCV for all traits could be due to the influence of environment on these traits. Panjeshahin et al. (2018) examined the genetic and morphological diversity of *M. longifolia* and found that PCV was higher than

GCV for leaf area, shoot length, and the number of primary branches.

Principal component analysis

PCA (principal component analysis) was applied to estimate the likely similarities and relationships between all populations of mint and all traits. PC1, PC2, and PC3 described most of the variation (80.34%) in the intraspecific cross of *M. longifolia* (Fig. 4). The first PC showed 48.3% of the total variation and had a positive correlation with essential oil yield (0.35), piperitenone (0.32), α -myrcene (0.18),

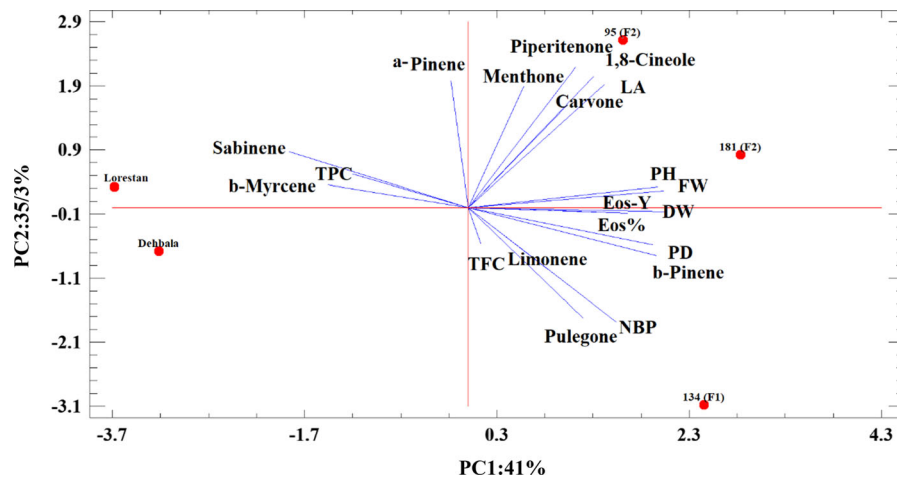


Fig. 5 PCA analysis for the classification of selected F_1 and F_2 genotypes of mint along with their parents based on growth traits and secondary metabolites in *M. spicata*. Lorestan: Parental clone, Dehbala: Parental clone, #134 (selected genotype of the F_1 generation): #95 and #181 (selected genotypes of the F_2 generation). PH: Plant height, NBP: Number of branches

α -pinene (0.21), 1, 8-cineole (21%), fresh weight (0.32), dry weight (0.33), and flavonoid content (20%) but a negative correlation with α -pinene (-0.30), menthone (-0.31) and pulegone (0.37). PC2 described 25.04% of the total variance and revealed a negative correlation with carvone (-0.34), sabinene (-0.29), and limonene (-0.19) but had a positive correlation with phenolic content (0.34).

In the intraspecific cross of *M. spicata*, the first three principal components revealed the highest variation (82.47%). The first PC (PC1) showed 52% of the total variation (Fig. 5) and had a positive correlation with essential oil yield (0.48), fresh weight (0.40), dry weight (45%), α -pinene (0.30), carvone (0.18), 1,8-cineole (0.19) and limonene (0.21), but a negative correlation with phenolic content (-0.27), sabinene (-0.29) and α -myrcene (-0.30). PC2 described 22% of the total variance and revealed a negative correlation with flavonoid content (-0.27) but a positive correlation with α -myrcene (0.39), menthone (0.26), and piperitenone (0.22).

In this study, the selected genotype #33 and #34 from the R- F_2 population and the genotype #227 from the F_2 population belonging to *M. longifolia* were discriminated by higher amounts of the first component. The genotype #33 had the maximum percentage of 1,8-cineole (1.5), and the genotype #227 with 67.76% and the genotype #34 with 61.5% had the

plant⁻¹, PD: Plant canopy diameter, FW: Fresh weight, DW: Dry weight, LA: Leaf area, Eos %: Essential oil content, Eos-Y: Essential oil yield, TPC: Total phenolic content, TFC: Total flavonoid content, α -Pinene: α -pinene, b-Myrcene: β -Myrcene, b-Pinene: β -pinene

maximum percentage of piperitenone. Due to the negative correlation between some essential oil compositions with the second component, the selected genotype #399 from the F_1 population belonging to *M. longifolia* with the lowest amount of this component, had higher essential oil constituents including limonene (13.64), sabinene (0.83) and carvone (1.55). A similar trend was observed for the intraspecific cross of *M. spicata*. By this, the selected genotype #181 from F_2 population with the highest amount of the first component had the maximum percentage of carvone (38.27) and 1,8-cineole (1.89) and the selected genotype #95 from the F_2 population with the highest amount of the second component had the maximum percentage of piperitenone (3.19). The results confirmed that selection based on morphological and phytochemical characterizations has increased some of the essential oil components in segregating populations.

Conclusion

In this paper, we described that there was a broad range of variations for growth and secondary metabolites among mint populations derived from intraspecific crosses. The range in most of the traits in F_2 and F_1 was higher than that of the parents, which can be due to

the existence of transgressive segregation and heterosis. Analysis of variance showed significant differences between F_2 and $R-F_2$ for all traits. Also, a significant difference was observed between F_1 and $R-F_1$ for fresh weight and dry weight along with plant canopy diameter and essential oil yield. This difference probably indicated the presence of cytoplasmic effects among the progenies of the crosses. The mean of $R-F_1$ and $R-F_2$ populations derived from the intraspecific cross of *M. longifolia* and F_1 generation belonging to *M. spicata*, showed superior EO yields and plant fresh weight, suggesting that growing these populations instead of the original clones may be more economical for food industries. Although the average of phenolic content in F_1 and F_2 populations derived from the intraspecific cross of *M. spicata* was not higher than that of the parents, all F_2 individuals had higher flavonoid content than the superior parent. In the reciprocal cross of *M. longifolia*, some F_1 and F_2 genotypes also had higher amounts of phenolics and flavonoids compared with the best parent. Therefore, the results of this paper confirmed that selection based on morphological traits and phytochemical characteristics could help breeders to achieve some genotypes with high valuable essential oil compositions compared with their parental clones, which can be useful for their mass cultivation and their industrial uses.

Acknowledgements This study was funded by Isfahan University of Technology, Isfahan, Iran.

References

- Abebe T, Alamerew S, Tulu L (2017) Genetic variability, heritability and genetic advance for yield and its related traits in rainfed lowland rice (*Oryza sativa* L.) genotypes at Fogera and Pawe, Ethiopia. *Adv Crop Sci Tech* 5: 272
- Abotalebian M, Keramat J, Kadivar M, Ahmadi F, Abdinian M (2016) Comparison of total phenolic and antioxidant activity of different *Mentha spicata* and *M. longifolia* accessions. *Ann Agric Sci* 61:175–179
- Adams RP (2005) Identification of essential oil components by gas chromatography/quadrupole mass spectrometry. *J Am Soc Mass Spectrom* 16:1902–1903
- Benabdallah A, Rahmoune C, Boumendjel M, Aissi O, Messaoud C (2016) Total phenolic content and antioxidant activity of six wild *Mentha* species (Lamiaceae) from northeast of Algeria. *Asian Pac J Trop Biomed* 6(9):760–766
- Bonea D, Urechean V (2003) Reciprocal cross effects for grain yield and content of raw protein in the maize grain. *Maize Genet Newsl* 77:67
- Brahmi F, Madani K, Stevigny C, Chibane M, Duez P (2014) Algerian mint species: HPTLC quantitative determination of rosmarinic acid and in vitro inhibitory effects on linoleic acid peroxidation. *J Coast Life Med* 2(12):986–992
- Brenner S, Miller JH (2001) *Encyclopedia of genetics*. Academic Press. 1631-1632, USA. ISBN: 9780122270802
- Burton GW, DeVane EH (1953) Estimating heritability in tall fescue (*Festuca arundinaceae*) from replicated clonal materials. *Agron J* 45:478–481
- Crocchi C, Asbach J, Novak J, Gershenzon J, Degenhardt J (2010) Terpene synthases of oregano (*Origanum vulgare* L.) and their roles in the pathway and regulation of terpene biosynthesis. *Plant Mol Biol* 73:587–603
- Diop SM, Guèye MT, Ndiaye I, Ndiaye EHB, Diop MB, Heuskin S, Lognay G (2016) Chemical composition of essential oils and floral waters of *Mentha longifolia* (L.) Huds. From Senegal. *Am J Essent Oil Nat Prod* 4:46–49
- Elansary HO, Yessoufou K, Shokralla S, Mahmoud EA, Skalicka-Woźniak K (2016) Enhancing mint and basil oil composition and antibacterial activity using seaweed extracts. *Ind Crops Prod* 92:50–56
- Ellstrand NC, Schierenbeck KA (2006) Hybridization as a stimulus for the evolution of invasiveness in plants? *Euphytica* 148:35–46
- Ene CO, Ogbonna PE, Ugwu Agbo C, Chukwudi UP (2019) Heterosis and combining ability in cucumber (*Cucumis sativus* L.). *Inf Process Agric*;6(1):150–157
- Fahlen A, Welander M, Wennersten R (1997) Effects of light-temperature regimes on plant growth and essential oil yield of selected aromatic plants. *J Sci Food Agric* 73:111–119
- Gharibi S, Tabatabaei BES, Saeidi G (2015) Comparison of essential oil composition, flavonoid content and antioxidant activity in eight *Achillea* species. *J Essent Oil Bear Pl* 18:1382–1394
- Golabadi M, Golkar P, Eghtedary AR (2015) Combining ability analysis of fruit yield and morphological traits in greenhouse cucumber (*Cucumis sativus* L.). *Can J Plant Sci* 95(2):377–385
- Guindon FM, Martin E, Cravero V, Cointy E (2019) Transgressive segregate ion, heterosis and heritability for yield-related traits in a segregation population of *Pisum Sativum* L. *Expl Agric* 55(4):610–620
- Gyenesne Hegyi Z, Kizmus L, Záborszky S, Marton LC (2001) Trends in the protein and oil contents and thousand kernel mass of maize under various ecological conditions. *Novenytermeles* 50:385–394
- Heydarzadeh P, Zahedi M, Sabzalian MR, Ataii E (2013) Mycorrhizal infection, essential oil content and morphological characteristics variability in three mint species. *Sci Hortic* 153:136–142
- Joshi HK, Chauhan DA, Pathakm AR, Viradiya YA (2016) Heterosis for quality parameters in direct and reciprocal crosses of dual purpose pigeon pea. *J Plant Breed* 7(4):1169–1178
- Kang MS, Mille JD, Tai PYP (1983) Genetic and phenotypic path analysis and heritability in sugarcane. *Crop Sci* 23:643–647
- Khanuja SPS, Shasany AK, Srivastava A, Kumar, (2000) Assessment of relationships in *Mentha* species. *Euphytica* 111:121–125

- Koide Y, Sakaguchi S, Uchiyama T, Ota Y, Tezuka A, Nagano AJ, Ishiguro S, Takamura I, Kishima Y (2019) Genetic properties responsible for the transgressive segregation of days to heading in rice. *Gen Soc of Am* 5:1655–1662
- Koseoglu K, Adak A, Sari D, Sari HF, Ceylan O, Tokar C (2017) Transgressive segregations for yield criteria in reciprocal interspecific crosses between *Cicer arietinum* L. and *C. reticulatum* Ladiz. *Euphytica* 213: 116
- Kumar B, Kumar U, Kumar Yadav H (2015) Identification of EST-SSRs and molecular diversity analysis in *Mentha piperita*. *Crop J* 3:335–342
- Kumar B, Mali H, Gupta E (2014) Genetic variability, character association, and path analysis for economic traits in Menthofuran rich half-sib seed progeny of *Mentha piperita* L. *BioMed Res Int*. <https://doi.org/10.1155/2014/150830>
- Kumar NVM, Katageri IS, Sunil Kumar GS, Gowda SA (2018) Transgressive segregation for fibre quality, seed cotton yield and its component traits in *Gossypium barbadense* L. *Cotton. Int J Curr Microbiol App Sci* 7(1); 1040-1051
- Kumar P, Mishra Y (2004) Genetic variability in wheat (*Triticum aestivum* L.). Biodiversity and sustainable utilization of biological resources. In: Proceedings of a National conference, Sagar, Madhya, Pradesh, India, pp 144–149
- Kumar S, Dwivedi VK, Tyagi NK (2003) Genetic variability in some metric traits and its contribution to yield in wheat (*Triticum aestivum* L.). *Progress Agric* 3:152–153
- Larik AS, Ansari SR, Kumbhar MB (1997) Heritability analysis of yield and quality components in *Gossypium hirsutum* L. *Pakistan J Bot* 29(1):97–101
- Mahboubi M (2018) *Mentha spicata* L. essential oil, phytochemistry and its effectiveness in flatulence. *J Tradit Complement Med* 1–7
- Manju PR, Sreelathakumary I (2002) Genetic variability, heritability and genetic advance in hot chilli (*Capsicum chinense* Jacq). *J Trop Agric* 4: 4–6
- Meier C, Mediavilla V (1998) Factors influencing the yield and the quality of hemp (*Cannabis sativa* L.) essential oil. *J Int Hemp Assoc* 5:16–20
- Murray MJ, Lincoln DE, Marble PM (1972) Oil composition of *Mentha aquatica* × *M. spicata* F₁ hybrids in relation to the origin of × *M. piperita*. *Can J Genet Cytol* 14:13–29
- Nazem V, Sabzalian MR, Saeidi G, Rahimmalek M (2019) Essential oil yield and composition and secondary metabolites in self-and open-pollinated populations of mint (*Mentha* spp.). *J Ind Crop Prod* 130:332–340
- Panjeshahin Z, Sharifi-Sirchi GR, Samsampour D (2018) Genetic and morphological diversity of wild mint *Mentha longifolia* (L.) Hudson subsp. noeana (Briq.) Briq. in South and Southeastern Iran. *J Med Plants By-prod* 1:105–115
- Pekal A, Pyrzynska K (2014) Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Anal Methods* 7:1776–1782
- Pinelo M, Rubilar M, Sineiro J, Nunez MJ (2004) Extraction of antioxidant phenolics from almond hulls (*Prunus amygdalus*) and pine sawdust (*Pinus pinaster*). *Food Chem* 85:267–273
- Riahi L, Elferchichi Ghazghazi H, Jebali J, Ziadi S, Aouadhi C, Chograni H, Zaouali Y, Zoghalmi N, Mliki A (2013) Phytochemistry: antioxidant and antimicrobial activities of the essential oils of *Mentha rotundifolia* L. in Tunisia. *J Ind Crop Prod* 49:883–889
- Rieseberg L, Archer M, Wayne R (1999) Transgressive segregation, adaptation and speciation. *Heredity* 83:363–372
- Saric-Kundalic B, Fialova S, Dobes C, Olzant S, Tekelova D, Grancai D (2009) Multivariate numerical taxonomy of *Mentha* species hybrids varieties and cultivars. *Sci Pharm* 77:851–876
- Scowcroft WR, Larkin PJ (1982) Somaclonal variation: a new option for plant improvement. In: Vasil IK, Scowcroft WR, Frey KJ (eds) Plant improvement and somatic cell genetics. Academic Press, New York, pp 159–178
- Smolik M, Rzepka-Plevens D, Jadcak D, Sękowska VA (2007) Morphological and genetic variability of chosen *Mentha* species. *Herba Pol* 53(3):90–97
- Srivastava NK, Misra A, Sharma S (2003) Variation among commercial cultivars of Japanese mint (*Mentha arvensis* L.) in the morphological and metabolite characters associated with essential oil yield. *J Hort Sci Biotechnol*; 78 (2): 154–160
- Talebi SF, Saharkhiz MJ, Kermani MJ, Sharafi Y, Fard FR (2016) Effect of ploidy level on the nuclear genome content and essential oil composition of Anise Hyssop (*Agastache foeniculum* [Pursh.] Kuntze). *Anal Chem Letters* 6:678–687
- Tucker A, Hendriks OH, Bos R, Fairbrothers DE (1991) The origin of *Mentha* × *gracilis* (Lamiaceae). II. Essential Oils. *Econ Bot* 45:200–215
- Tucker AO, Chambers HL (2002) *Mentha canadensis* L. (Lamiaceae): a relict amphidiploid from the Lower Tertiary. *Taxon* 51:703–718
- Tucker AO, Fairbrothers DE (1990) The origin of *Mentha* × *gracilis* (Lamiaceae). I. Chromosome numbers, fertility, and three morphological characters. *Econ Bot* 44:183–213
- Yaghini H, Sabzalian MR, Rahimmalek M, Garavand T, Maleki A, Mirlohi A (2020) Seed set in inter specific crosses of male sterile *Mentha spicata* with *Mentha longifolia*. *Euphytica* 216:46
- Yahia IBH, Bouslimi W, Messaoud C, Jaouadi R, Boussaid M, Zaouali Y (2018) Comparative evaluation of Tunisian *Mentha* L. species essential oils: selection of potential antioxidant and antimicrobial agents. *J Essent Oil Res* 31:184–195
- Zhang DY, Yao XH, Duan MH, Wei FY, Wu GH, Li L (2015) Variation of essential oil content and antioxidant activity of *Lonicera* species in different sites of China. *Ind Crops Prod* 77:772–779

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.